Bioavailability of D_4 after Inhalation and Implantation Exposure to Silicones

In the November 2001 issue of *EHP*, Luu and Hutter (*1*) described a physiologically based pharmacokinetic (PBPK) model for the bioavailability of octamethylcyclotetrasiloxane (D_4) following exposure to D_4 by inhalation and implantation. In this paper the authors developed a PBPK model that used a very limited data set obtained after either single or repeated intravenous (iv) administration of D_4 as a microemulsion (*2*). The intravenous pharmacokinetic data reported by Kirkpatrick (*2*) were obtained from a study I helped design and conduct; I am familiar with the data and with the limitations of the study design for this type of assessment. Kirkpatrick (*2*) obtained blood and tissue samples at various time intervals after administration of radiolabeled D_4 and determined total radioactivity in these samples, but did not attempt to distinguish between parent D_4 and D_4 metabolites. Although the data obtained by Kirkpatrick were for iv dosing, Luu and Hutter (*1*) actually used intra-arterial dosing in their PBPK model. They validated their model by predicting inhalation kinetics in rats and comparing their prediction with a data set published by Plotzke et al. (*3*); they assumed that the radioactivity measured by Plotzke et al. (*3*) was parent D_4, with no contribution from metabolites. Luu and Hutter (*1*) plan to use their PBPK model to assess risk after exposure to D_4 resulting from migration from silicone gel breast implants. In addition to specific issues about their PBPK model, I also have several concerns about the manner in which this model will ultimately influence any risk assessment performed for D_4. These concerns relate to *a*) the assumptions of the level of D_4 in a silicone gel breast implant, *b*) the actual level of exposure to D_4 arising from a silicone gel breast implant, *c*) the limited understanding of the metabolism of D_4 reported by Luu and Hutter (*1*), and *d*) the prediction from their PBPK model that D_4 will bioaccumulate with repeated exposures.

The level of low molecular weight siloxanes (LMWS), both cyclic and linear, that persist in the polydimethylsiloxane (PDMS) used to make the silicone gel and elastomer shell of a breast implant is in the range of ≤ 0.1%. In a recent comprehensive pharmacokinetic study on PDMS, Jovanovic (*4*) measured the actual concentration of D_4 to be 0.03% of the PDMS by weight. Our own analysis of D_4 in silicone gel breast implants shows that D_4 levels rarely exceed 700–1,000 ppm (0.07–0.1%) (*5*). This higher level of D_4 in the silicone gel could result during the manufacturing process. If one conservatively assumes that a silicone gel breast implant could contain up to 0.1% D_4 and that the average size of a breast implant is 250 g, then the total D_4 content in two breast implants is 500 mg, or 8.7 mg D_4/kg body weight based on the U.S. Environmental Protection Agency's default body weight of 57 kg for a woman (*6*).

The migration of silicone from a silicone gel breast implant ranges up to 820 µg/day (*7*), with the migration of D_4 occurring at a rate of about 0.58 µg/day (*5*). For a woman who weighs 57 kg, this migration equates to a relatively small exposure of 0.01 µg/kg/day. Luu and Hutter (*1*) estimated that the extra dose of D_4 received from a silicone gel breast implant is 5.7 µg/kg/day, an overestimate by over 500-fold. The estimate of daily intake reported by Shipp et al. (*8*) resulting from exposure to D_4 in a wide variety of personal care products was 158 µg/kg/day. If we assume the value reported by Luu and Hutter (5.7 µg/kg/day) is correct, then the exposure to D_4 resulting from migration from a gel-filled implant would account for a proportionately small increase in total exposure to D_4 (from 158 µg/kg/day to 164 µg/kg/day). This small increase has little effect on the initial risk assessment for D_4 (*8*).

Two of the references (*9,10*) cited by Luu and Hutter (*1*) to support "migration of significant amounts of silicone out of gel implants into surrounding tissue and to the liver" have been retracted by the authors (*11*). Further, Hull (*12*), a member of the *Magnetic Resonance in Medicine*'s Editorial board, wrote that "as a referee, none of Garrido's papers should have been published in their current form," and in a summary statement concluded that

> the inadequacies, omissions, inconsistencies, and unresolved questions that are apparent in the work of Garrido et al. allow only one possible conclusion: there is no convincing and reproducible evidence of millimolar concentrations of silicon in tissue or blood.

The work of Garrido and colleagues (*9,10*) certainly does not support the contention of Luu and Hutter (*1*) in the introduction of their paper that

> the migration of significant amounts of LMWS from silicone gel breast implants … would add to the dermal or inhalation exposures from personal care products in a typical woman.

Luu and Hutter (*1*) postulated that D_4 saturates the elimination process, thereby potentially increasing the delivered dose to the target tissue and causing accumulation of D_4 in fat, liver, and kidneys. This conclusion is based on their analysis of the iv data (but they actually used intra-arterial administration). Several studies show that D_4 induces cytochrome P450 2B1/2B2 in a time, dose-dependent, and phenobarbital-like manner (*13,14*). Studies conducted by Plotzke and colleagues (*3,15,16*) and Varaprath et al. (*17,18*) provide evidence that rats extensively metabolize D_4. Metabolism and subsequent elimination of hydrophilic metabolites in urine and feces are important elimination mechanisms for D_4 in mammalian species. In addition, the elimination of D_4 occurs not only by this high metabolic clearance from liver but also by exhalation of parent D_4 via the lung. If Luu and Hutter (*1*) were correct and D_4 did saturate the enzymes responsible for metabolism, proportionately more D_4 would be eliminated through exhalation. As shown by Plotzke et al. (*3,15,16*), in fact, the rates of metabolism and clearance of D_4 and its metabolites support the conclusions reached with a more comprehensive PBPK model developed by Andersen et al. (*19*); that is, D_4 will not be unusually persistent in mammalian species.

In their discussion, Luu and Hutter (*1*) focused much of their attention on the potential bioaccumulation of D_4. The PBPK model developed by Andersen et al. (*19*) was based on an extremely robust inhalation pharmacokinetic data set for D_4 developed by Plotzke et al. (*3*) that included exposure to three concentrations, single and repeated exposures, and separate measurement of parent D_4 and metabolites (*15–18*). This model showed that D_4 is not expected to accumulate with repeated exposures. This lack of accumulation, despite high fat:blood partitioning, is due to rapid metabolism and the low blood:air partition coefficient that allows for ready exhalation of D_4. Metabolism does not saturate until the inhalation exposure concentration exceeds 500 ppm (v/v). To assess the validity of the prediction that D_4 would not accumulate, we recently collected blood and fat samples from female rats after 6 months of exposure to D_4. As part of a 2-year bioassay, these female rats were exposed by inhalation for 6 hr/day, 5 days/week to 700 ppm (v/v) D_4. We measured parent D_4 concentrations in both the blood and fat and compared the concentrations at 6 months of exposure with those obtained at 15 days in the inhalation pharmacokinetic study by Plotzke et al. (*3*). The concentrations in blood and fat, respectively, at 15 days were 7.2 µg/g and 1,079 µg/g tissue. At 6 months, the D_4 concentrations in blood and fat, respectively, were 13 µg/g and 1,200 µg/g tissue. These results confirm that D_4 does not accumulate in the body.

As with any risk assessment, it is essential to understand both the exposure to target

populations and the dose response for toxicity in experimental animals. The development of a PBPK model plays an important role in calculating the dose delivered to target tissue from specific exposure conditions. These PBPK models also can play a role in understanding the dynamic processes that occur while the D_4 is in the organism. Recently, D_4 was shown to have an effect on the reproductive system of female rats following inhalation exposure to 500 and 700 ppm (v/v) (*20*). This effect consisted of a reduction in mean live litter size and implantation sites. In the F_1 generation, there also was a reduction in mating at 500 and 700 ppm (*20*). The mode-of-action for these reproductive effects is the ability of D_4 to block or shift the preovulatory surge of luteinizing hormone (*21*). The highest exposure concentration that does not cause a significant reproductive effect [i.e., the no-observed-adverse-effect level (NOAEL)] appears to be around 300 ppm. The estimate of daily intake reported by Shipp et al. (*8*) for D_4 exposure from a variety of sources including personal care products is influenced by two characteristics or assumptions. First, at the time we completed our initial exposure assessment, roll-on antiperspirants (AP) contained up to 60% D_4 and accounted for about 50% (70 µg/kg/day) of the estimated daily intake. In the last few years, there has been a shift away from D_4 in roll-on APs such that the estimate of daily intake today should be about 40–50% lower than the original value. Second, the primary exposure to D_4 in personal care products is dermal application. After absorption into the venous blood, D_4 goes to the lung before reaching other tissues. As D_4 passes through the lung, some is eliminated in the expired air before entering the arterial circulation. Based on its partition coefficient, one-half of the free D_4 in the venous blood will be exhaled during passage through the lung. This first pass effect, predicted by the PBPK model developed by Andersen et al. (*19*) is consistent with the physical properties of D_4 and therefore further lowers the estimated daily intake. Luu and Hutton (*1*) estimated a daily intake or exposure resulting from migration of D_4 from a silicone gel breast implant to be 5.7 µg/kg/day, which is likely to significantly overestimate the actual daily intake. However, if we conservatively estimate the daily intake from personal care products to be 78 µg/kg/day (based on the reduced use of D_4 in roll-on APs as discussed above) and add the estimated daily intake or exposure by Luu and Hutter, then the estimated total daily intake for D_4 becomes 85 µg/kg/day. Exposure of rats to 300 ppm (v/v) of D_4 for 6 hr/day equates to an inhaled dose of 45,000 µg/kg/day using an absorption value of 5%, as determined in our inhalation pharmacokinetic studies (*3*). These values give a margin of safety (or exposure), as determined by dividing the NOAEL by the estimated daily intake, of over 500. A margin of exposure (MOE) of a specified magnitude indicates that exposure at or below the corresponding estimated intake level is not expected to result in adverse effects in the exposed populations. An MOE of 100 is typically considered large enough to be health protective when the NOAEL is based on animal data. The components of the MOE can be thought of as the typical factors of 10 for interspecies extrapolation (from animals to humans) and a factor of 10 for intrahuman variability, resulting in an MOE of 100.

In summary, Luu and Hutter (*1*) reported that they have developed a PBPK model for exposure to D_4 via two routes: *a*) inhalation in association with daily use of multiple personal care products, and *b*) migration of small amounts of silicone fluid from silicone gel breast implants. Their PBPK model is built from data generated by intravenous administration of D_4 as a microemulsion (*2*) and then modeled for intra-arterial dosing. They assumed that all radioactivity was parent D_4, even though there is significant conversion of D_4 to hydrophilic metabolites. A more complete PBPK model (*3*) was developed from an extensive inhalation data set on D_4, including evaluation of metabolism of D_4. This more comprehensive model and the actual data from our 6-month inhalation study show that there are only modest increases of D_4 concentration in fat on repeated exposures to D_4 compared to concentrations achieved after single exposures. Luu and Hutter (*1*) also overestimated the contributions to the daily intake resulting from the migration of D_4 from a breast implant. However, this overestimation of the daily intake by Luu and Hutter does not significantly change the MOE for D_4. The conservative MOE of > 500 indicates that current use practices with D_4 have adequate safety margins

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Further Comments on the Bioavailability of D_4

We would like to comment on a paper by Luu and Hutter (*1*) published in the November 2001 issue of *EHP*. We have developed multidose route, multi-species PBPK models for D_4 over the past several years. Our PBPK models have been presented in abstract form at several national meetings, and the complete inhalation model for D_4 in the rat was published earlier this year (*2*). In their paper, Luu and Hutter (*1*) incorrectly attribute several conclusions to our earlier abstracts, including the comment that our model did not describe blood concentrations during and after exposure. Surprisingly, they did not cite conclusions from our complete, peer-reviewed documentation of our model. We would like to point out some important differences between their model and our D_4 model. We would like to address several issues: *a*) the unconventional model structure and inappropriate use of available pharmacokinetic data to estimate the blood:air partition coefficient by Luu and Hutter (*1*); *b*) the process by which all available pharmacokinetic data should have been used to ensure adequate validation of their PBPK model; and *c*) the unusual kinetic behavior of D_4 compared to other volatile organic compounds that needs to be captured in any kinetic model for this compound.

A major difference in Luu and Hutter's model (*1*) and our published model (*2*) is the value used for the blood:air partition coefficient ($P_{b:a}$). Our estimate of $P_{b:a}$ derived from the measured concentrations of parent D_4 in blood at the end of a 6-hr exposure was 0.8; our direct measurements of the $P_{b:a}$ by equilibration of D_4 between blood and air *in vitro* gave a value near 4.0. Luu and Hutter used a much higher value of 20 and reported that they were able to describe both the rat and human inhalation results. It is of interest to determine why there would be such a large discrepancy in a critical parameter between the two models.

Luu and Hutter's model for D_4 in the rat (*1*) is based on studies in which total radioactivity was measured in blood after exposure of rats to ^{14}C-D_4. Luu and Hutter (*1*) used the radioactivity data from Plotzke et al. (*3*) and assumed that the radioactivity in blood was parent compound. In our work, we modeled parent D_4 and metabolites separately. By the end of the 6-hr inhalation exposure in rats, the majority of radioactivity in blood is metabolite (about a 3- to 4-fold greater concentration of metabolite vs. parent D_4 at the end of the exposure). After the 6-hr exposure, D_4 is rapidly eliminated by exhalation compared to the metabolites, and the discrepancy between total radioactivity and parent D_4 only increases. To predict these artificially high blood levels and retain these high concentrations for long periods of time, Luu and Hutter's model requires an artificially high estimate of the partition coefficient, thus the use of 20 in their model versus 1.0 in our model in which parent D_4 and metabolites were described separately.

Luu and Hutter (*1*) then scaled the model with the high partition coefficient to humans. In this case the data in their paper was for parent D_4; nonetheless, they still showed good correspondence between data and model predictions. We believe that this agreement is quite misleading and related to differences between their human modeling approach and conventional approaches used with other volatiles. Their ability to fit the human D_4 was based on an artificial constraint added to limit retention of inhaled D_4.

Based on the equations of Ramsey and Andersen (*4*), a paper cited as the basis of Luu and Hutter's work, the concentration of styrene in the arterial air (C_{art}) could be approximated from a steady-state formula published by Andersen (*5*):

$$C_{art} = \frac{P_{b:a} \times Q_{alv} \times C_{inh}}{Q_{alv} + P_{b:a} \times E_H \times Q_H}, \qquad [1]$$

where Q_{alv} is the alveolar ventilation, E_H is hepatic extraction, Q_H is the hepatic blood flow, and C_{inh} is the inhaled concentration of compound. In PBPK models, inputs include partition coefficients, inhaled concentrations, and the suite of physiologic factors, including blood flows, breathing rates, and characteristics of metabolizing tissues. Using all of these factors together, it is possible to predict the amount of inhaled compound that is retained during respiration. For modeling exposures in rats, Luu and Hutter (*1*) correctly used the ventilation × the inhaled concentration as the input term to the arterial blood in the rats. In contrast, for the human modeling Luu and Hutter (*1*) cited the differences (input – output) measured in a human study from the University of Rochester (*6*) and applied them as a constraint on the model. Thus, their input is ($Q_{alv} \times C_{inh} \times$ proportion retained). Because the proportion retained was only 0.1, the model required an anomalously high blood:air partition coefficient to achieve blood concentrations equal to the inhaled air concentrations. (This behavior follows from Equation 1 if the proportion retained is included empirically.) Our PBPK model for D_4, following previous approaches with volatile compounds such as styrene, describes parent D_4 concentrations in rat and humans without artificial constraints on uptake. The proportion retained is an output of the model, not a constraint. In this fashion, both rat and human uptake curves are adequately described in our modeling efforts with $P_{b:a} = 1.0$.

The novel kinetic behavior referenced in the title of our paper (*2*) is the persistence of nonexchangeable D_4 in blood at long times after exposure. We only identified the necessity to include this bound form in blood because of our efforts to fit blood and exhaled D_4 during both the exposure and the postexposure periods. Luu and Hutter's model (*1*) also included blood sequestration from the plasma pool of D_4. (The equation in their paper for the weakly bound compartment appears to be incorrect. The last term in their paper for this equation should be $k_{si} \times C_{wk}$ rather than $k_{si} \times C_{str}$. According to the author's description

$$V_{weak}\frac{dC_{wk}}{dt} = k_{wi}C_{ai} + k_{so}C_{str} - k_{wo}C_{wk} - k_{si}C_{str},$$

where C_{ai} is the concentration of D_4 dissolved in plasma; C_{wk} is the concentration of D_4 weakly protein bound in plasma; C_{str} is the concentration of D_4 strongly protein bound in plasma; k_{wi} is forward rate constant for weak protein binding of D_4 in plasma; k_{si} is forward rate constant for strong protein binding of D_4 in plasma; k_{so} is reverse rate constant for strong protein binding of D_4 in plasma; k_{wo} is reverse rate constant for weak protein binding of D_4 in plasma; V_{weak} is the volume of weakly bound plasma.

Another similarity in structure of the two models is the use of multiple fat compartments. Luu and Hutter (*1*) used a diffusional movement from a single fat compartment into a sequestered compartment within the main fat compartment. In our model, we described different fat compartments within the body with different time constants for equilibration. Luu and Hutter (*1*) referred to blood flow to deep fat, although the description and equations indicate a diffusional movement from weakly bound fat to the deep fat compartment. Their equation for the deep fat compartment is also inaccurate as written; it should show a term for movement from the weakly bound fat compartment. In its present form in their paper (*1*), the rate of change of mass for the deep fat would always be zero. [The equation for the lung compartment in Luu and Hutter's paper (*1*) also has an error, with C_{lung} appearing twice in the second term of the mass balance equation.]

The model structure used by Luu and Hutter (*1*) for intravenous dosing actually is for intra-arterial dosing, in which the compound is placed in the arterial blood and

infused into tissues rather than introduced into the venous blood, where it must traverse the lung with opportunity for exhalation before passing to the arterial blood. For a compound with a low $P_{b:a}$ such as D_4, it is important to have physiologic realism in the dosing route in order to estimate exhaled D_4 accurately after intravenous dosing.

Another issue is that Luu and Hutter (*1*) should have used all available pharmacokinetic data to insure adequate validation of their PBPK model. After configuring the model for intravenous dosing, a practice common to many pharmacokinetic studies, Luu and Hutter (*1*) predicted plasma and fat concentrations for a single inhalation exposure of rats to D_4. The model overestimated the early time points in fat. In addition, the overall time course in plasma was underestimated for this one attempt at extrapolation and validation. Surprisingly, this validation exercise used a single study from an extremely rich data set on the inhalation pharmacokinetics of D_4 in rats. The data used for dose route extrapolation and validation once again were for radioactivity rather than for parent D_4 in blood and fat, whereas their pharmacokinetic model was purportedly for parent D_4 alone.

Plotzke et al. (*3*) performed pharmacokinetic studies of inhaled D_4 in male and female rats at three exposure concentrations for both single and multiple exposures. These inhalation studies generated important data on tissue time courses of D_4 in a large set of tissues, as well as in exhaled breath concentrations. Similarly, the available human data for interspecies extrapolation include exhaled breath concentrations and blood concentrations from volunteers (*6*). Any model validation exercise should consider all available kinetic information and not rely on a limited selection of these results. Luu and Hutter's (*1*) conclusions regarding validation should be regarded as preliminary until their PBPK model is rigorously tested against more complete data sets. For Luu and Hutter to assert that prediction of a limited set of available human data from an unconventional model for inhalation constitutes dose–route and interspecies validation of their PBPK model is an overinterpretation of available information.

A third area of concern in Luu and Hutter's study (*1*) involves the unusual kinetic characteristics of D_4. There is little doubt that the defining characteristic of D_4 is its lipophilicity, including a high fat:blood partition coefficient (P_f). We determined by vial equilibration methods that P_f was 500–600 in rats (*2*). The overall kinetic behavior of D_4, however, is related to several important characteristics: lipophilicity, high metabolic clearance from liver, and high exhalation clearance due to its relatively low $P_{b:a}$. This suite of characteristics insures that D_4 does not bioaccumulate excessively with repeated dosing. Although both Luu and Hutter's model (*1*) and our PBPK model agree that the fat–time constant is of the order of several weeks, D_4 behaves much differently from poorly metabolized, nonvolatile compounds that bioaccumulate extensively with multiple exposures. The blood levels of D_4 do not increase with daily exposures and the fat concentration increases only slightly, as noted in the multiple exposure studies reported by Dow Corning scientists and analyzed with our more complete PBPK model (*2*). On a fairly minor note, the pharmacokinetic models developed by both groups are linear, low-dose models. Luu and Hutter (*1*) called the kinetics of the intravenous administration nonlinear. The appropriate terminology would be polyexponential, not nonlinear.

We are pleased to see PBPK modeling approaches for evaluating interspecies differences in disposition appear in *EHP;* however, Luu and Hutter's statements regarding our inability to model postexposure D_4 levels are inaccurate. The postexposure kinetic behavior of D_4 is determined by a combination of free D_4 and D_4 in a nonexchangeable compartment. These time-course curves have been accurately described at various concentrations after both single and multiple exposures in male and female rats with our PBPK model structure (*2*). As Luu and Hutter noted, we did not report extrapolation to humans. The reason for this was that we were in the process of completing a more definitive examination of human inhalation kinetics from two complete human data sets on a total of 18 exposures. These analyses have now been completed (*7,8*).

To summarize our human modeling, we found that the structure of the rat PBPK model for D_4 with a $P_{b:a}$ of near 1.0, when scaled appropriately, was entirely adequate for describing all available data from human volunteers. We are concerned about the inaccurate attribution of conclusions of our modeling efforts by Luu and Hutter (*1*) and appreciate the opportunity to provide clarification on these points. We emphasize that the kinetics of D_4 are well described with $P_{b:a}$ = 1.0 in both rats and humans, when sequestration in blood lipids is included in the model structure. Because of the high rate of metabolism and exhalation of poorly soluble D_4 from blood, there should be little tendency for D_4 to bioaccumulate in any tissues upon repeated exposures.

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Rebuttal and Critical Review of Andersen et al.'s D_4 PBPK Model

The letters of Meeks and Andersen et al. regarding our paper in *EHP* (*1*) included inaccurate statements and misconceptions about our pharmacokinetic model of D_4.

After reviewing Andersen et al.'s recent paper (*2*), we found several shortcomings. First, Anderson et al. (*2*) used an unconventional experimental method to underestimate the affinity of D_4 for blood and fat; these partition coefficients were not comparable to those obtained for other lipid soluble organic chemicals. They further reduced

these experimental measurements in order to "fit" a 10-compartment model, which included 3 deep compartments in the lungs, fat, and liver. Andersen et al. used these low values to underestimate potential D_4 accumulation in fat and increase its clearance.

Second, Andersen et al. (*2*) did not validate and verify their PBPK model using independent data from intravenously (iv) treated rats. When we used Andersen et al.'s parameters for D_4 [blood:air partition coefficient ($P_{b:a}$), fat:blood partition coefficient (P_{fat}), and metabolism rate (V_{max})] in our own model, our results did not fit the iv experimental rat data, especially regarding D_4 tissue distribution in fatty tissues. Andersen et al.'s conclusions about the disposition and fate of D_4 also were not substantiated by the experimental rat inhalation data because high lipid solubility and slow desorption would favor accumulation in fatty tissues, as in the case with styrene.

Third, in their letter, Andersen et al.'s criticism about the dose rate of D_4 from a breast implant was incorrect; the dose rate reported was for D_4 leaching from the saline-filled breast implants and not from the silicone gel-filled breast implants.

We question the validity of Andersen et al.'s model (*2*) and believe that their predictions about the safety assessment of D_4, a component in silicone personal products and breast implants, may be misleading.

Andersen et al. (*2*) used a low $P_{b:a}$ (0.88) in their model, despite reporting a measured experimental value of 4.3. They also used an unconventional method to measure the $P_{b:a}$ and blood:tissue partition coefficient ($P_{b:t}$).

To measure the $P_{b:a}$ and $P_{b:t}$ of D_4, Andersen et al. (*2*) placed liquid D_4 and matrices such as blood, fat, lung, and liver in separate glass scintillation vials. All of the vials were subsequently placed in an enclosed 500-mL beaker. The D_4 was not in physical contact with the blood or any other matrices throughout the experiment. Using this method, a low volatility compound like D_4 would have to vaporize, diffuse through a gas space, and diffuse into a stagnant blood or tissue phase with liquid mass transfer resistance. This process would take time to reach equilibrium, but did Andersen et al. allow enough time for equilibrium to occur? Shields et al. (*3*), who measured D_4 concentrations in indoor air using a state-of-the-art analytic method, indicated that the sampling intervals for D_4 should be in weeks, not hours, in order to reach equilibrium. Andersen et al. (*2*) reported that they agitated for 24 or 48 hr and measured $P_{b:a}$ at two unknown time points. In fact, if the samples were allowed to reach equilibrium, their measurement of the $P_{b:a}$ of D_4 (4.3) might reach our estimated value of 20. The measured concentrations of D_4 in blood based on molecular diffusion between the vapor phase of D_4 and blood are not reliable unless they used long sampling intervals (*3*). Because Andersen et al. (*2*) did not describe internal standards for the experiment, it is likely that the percentage recovery was low after 24–48 hr. The same method was also used to underestimate other partition coefficients for fat, lungs, and kidneys.

A more accurate and direct measurement of $P_{b:a}$ (or $P_{tissues}$) would be to place several milliliters of the viscous D_4 liquid in direct contact with the tested matrix (e.g., whole blood, fat, liver, etc.) in a closed scintillation vial (*4*). The headspace (air) concentration and matrix concentration of D_4 should then be quantified during several time intervals following agitation. This minimizes the equilibrium problems not addressed by Andersen et al. (*2*).

The physical properties of D_4 (Table 1) play an important role in its tissue distribution and excretion; thus it is important that the use of arbitrary "fitted" parameters be avoided. This arbitrary low value of $P_{b:a}$ used by Andersen et al. (*2*) differed by a factor of 5 from the *in vitro* evaluation. Similarly, the partition coefficients used for fat and other tissues also varied widely from their experimental data (*2*). For example, Andersen et al. used a P_{fat} of 550.6 for instead of their experimentally determined value of 2,089 so their model would fit the data.

The low $P_{b:a}$ value is not comparable to those of other organic chemicals with properties similar to those of D_4. As shown in Table 1, the higher the volatility, the smaller the value of $P_{b:a}$ of an organic compound. For example, because benzene is more volatile than styrene, it has a smaller $P_{b:a}$ (75% smaller) than styrene (Table 1). According to Andersen et al.'s results (*2*), D_4 would be more volatile than benzene in blood. This is inconsistent with the observed volatility because benzene has a boiling point of 80.1°C, whereas D_4 has a boiling point of 175°C (Table 1). Because D_4 has a lower volatility than both styrene and benzene, its $P_{b:a}$ would be expected to be at least as large as the values reported for these two chemicals, and not smaller (Table 2). The D_4 $P_{b:a}$ would not be expected to have a value as low as 0.88, which is outside the range of all of the chemicals listed in Table 2. Ramsey and Andersen (*16*) reported a $P_{b:a}$ for styrene of 40.2 (Table 1).

Under the scenario of Andersen et al. (*2*), if both 1 µg D_4 and 1 µg of a much more volatile component such as benzene or another chemicals in Table 2 were added to blood, the D_4 would vaporize more readily. This is due to its partition coefficient, which favors transfer to the gas phase. Thus, D_4, which boils at 175°C, would be more volatile than benzene, which boils at 80.1°C, a situation which makes no sense.

As we discussed in our paper (*1*), the physical properties of D_4 favored its absorption into fat. High absorption of D_4 (100%) by the iv route and slow desorption, as well as a long half-life in fat ($t_{1/2}$ = 18 days), were attributable to the high $P_{b:a}$, P_{fat}, and high lipid solubility of D_4 [log octanol/water partition coefficient (K_{ow}) = 5.1]. For similar reasons, other highly lipid soluble organic compounds such as styrene, with high $P_{b:a}$ and P_{fat}, tend to accumulate in the fat tissue of rats and humans (*5–7*).

To compensate for this estimate of a thermodynamic property in blood and to "fit" the rat data for inhalation exposure, Andersen et al. (*2*) modified their basic model with 6 compartments to a refined model with 10 compartments, including deep compartments (deep lung, deep liver,

Table 1. Comparison of physical properties of D_4, styrene, and benzene.

Property	D_4	Styrene	Benzene
Melting point (°C)	17.5	–30.6	5.5
Boiling point (°C)	175.4	145–146	80.1
Vapor pressure (mmHg)	1 (25°C)	4.5 (20°C)	2.3 (3°C)
$P_{b:a}$	0.88[a]	40–52	17.8
Solubility in water	56 ppb	300 ppm	—
Log K_{ow}	5.1	2.95	2.14

[a]We used a value of 20 for $P_{b:a}$.

Table 2. Blood:air ($P_{b:a}$) and blood:fat partition coefficients (P_{fat}) of some known VOCs.

Compound	Log K_{ow}	P_{fat}	$P_{b:a}$
Hexane	3.87	69.43	2.29
Isoprene	2.42	38.5	1.87
1,1,1-Trichloroethane	2.48	45.66	5.76
Tetrachloroethene	3.40	86.67	18.9
Benzene	2.14	28.03	17.8
Toluene	2.64	56.72	18
p-Xylene	3.15	42.32	41.3
Styrene	2.95	86.47	40.2
Chlorobenzene	2.86	21.5	59.4

deep fat). But any scenario can be fitted by simply adding more compartments. However, adding more mass balance equations requires more biochemical parameters, which may not be available or accurately measured.

In our study, we derived the $P_{b:a}$ as the reciprocal of the D_4 Henry's Law Constant, which is its published water:air partition coefficient (a value ranging from 3 to 32) (*8–11*). The value we used in our model was within the range reported by these independent investigators (*8–11*). Still, we included in our paper (*1*) a discussion of the discrepancy caused by blood to the aqueous Henry's Law Constant of D_4, and we also cited the paper that supported these observations (*4*). We believe that Andersen et al. (*2*) did not take into account the $P_{b:a}$ of lipophilic organic compounds described by Beliveau and Krishnan (*4*).

Even though our $P_{b:a}$ is significantly larger than that reported by Andersen et al. (*2*) we predicted that the absorbed D_4 would be mostly exhaled [range, 42–59% in humans; see Table 6 of our paper (*1*)]. We do not understand Andersen et al.'s comment in their letter that we did not predict significant exhaled D_4. Recalculating the exhaled air amount using the following material balance on the exhaled air may clarify our concerns to Andersen et al.

$$\text{Exhaled} = \int_0^t Q_{air} C_{air} dt \qquad [1]$$

In preparing this response, we ran our PBPK model again using Andersen et al.'s fitted values (*2*) for $P_{b:a}$, P_{fat}, and V_{max} for metabolism rate (8 times higher than our V_{max} value). The results in Figure 1 show that the model using parameters employed by Andersen et al. (*2*) predicted poorly the D_4 levels in fat while predicting reasonable plasma D_4 levels following a single, low-dose iv injection. Using Andersen et al.'s parameters (*2*), we found that > 80% of D_4 is exhaled after iv exposure. Therefore, this would cause underprediction of D_4 accumulation in fat, experimentally found to be 16% of the iv dose. As shown in Figure 5 in our paper (*1*), our model gave excellent simulations of the rat iv (*12*) and rat inhalation data (*13*).

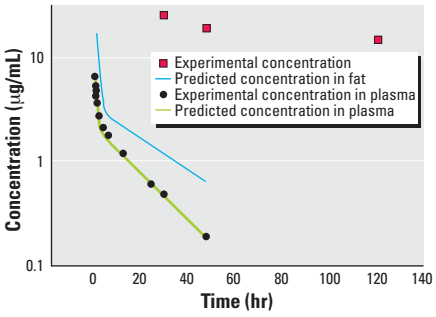

Figure 1. Comparison of predicted and experimental D_4 concentrations in fat and plasma. The experimental concentration in fat is from our model (*1*), and other values are from Andersen et al. (*2*). Andersen et al.'s model underpredicts the accumulation of D_4 in fat.

Two structurally different PBPK models could not both be correct, and both models fit the rat inhalation reasonably well. This leads us to believe that there are other shortcomings in Andersen et al.'s study (*2*):

- Andersen et al. (*2*) based their model on a rat inhalation study in which the absorption and elimination rates are compromised. Only 10% of the exposed D_4 in the air is absorbed, compared to 100% absorption of D_4 with iv exposure. The dose absorption is limited from the mass transfer resistance in the lungs. Using a low $P_{b:a}$, Andersen et al. reported that > 50% of the D_4 absorbed is eliminated in the expired air, whereas they assumed the metabolism rate of D_4 to be 8 times higher in their model than in ours. The unusual kinetics could not be confirmed by other published studies.
- Andersen et al.'s model lacks validation and verification using independent data such as included in the rat iv study (*12*), so their conclusions about the disposition of D_4 are best described as preliminary.
- Andersen et al.'s model is not accurate because they failed to measure partition coefficients for both parent compounds and metabolites for the 10 compartments including 3 deep compartments (lungs, liver, and fat). Instead, they have to curve fit, leading to errors and uncertainty regarding D_4 distribution in fatty tissues especially.
- Andersen et al.'s conclusions on D_4 kinetics even contradicted what others (*13,14*) reported regarding D_4 kinetics. In fact, they reported that D_4 plasma and tissue distributions resemble other volatile organic compounds such as styrene, which were found to accumulate in fat tissues of both experimental animals and exposed workers (*5–7*).
- Andersen et al. (*2*) failed to determine accurate D_4 pharmacokinetic data which show that D_4 is retained in fat. Because 8–10% of D_4 dose was found in fat 7 days postexposure and because rats were to be exposed daily for 14 days, it is hard to believe that D_4 would not retain and accumulate in the body.

In our paper (*1*), we estimated the maximum dose rate of residual D_4 that could migrate from the silicone envelope of a breast implant to be 5.7 µg/kg/day based on Fick's Law of Diffusion. We estimated a leaching rate of 95% in 30 days for the thin shell of a saline-filled breast implant surrounded by fatty tissues. The diffusivity of 5.4×10^{-8} cm^2/sec was consistent with published values for other chemicals (*15*). Our reported dose rate was the dose rate of D_4 leaching from saline-filled breast implants. The dose rate of D_4 leaching out of implanted silicone gel-filled breast implants could be easily determined, if needed.

In their letter, Andersen et al. correctly identified a typographical error in the Appendix regarding the material balance on the lung. The correct equation is as follows:

$$V_{lung} \frac{dC_{lung}}{dt} = Q_t C_{ai} - Q_t C_{lung} H_{air} - Q_{air} C_{lung}$$

In this equation, C_{ai} is a venous blood concentration as defined by the equation at the mix point [Appendix of our paper (*1*)]. It is not an arterial concentration, as suggested by Andersen et al. In this nomenclature, a = average. Thus, unlike the claims of Andersen et al. in their letter, this model does not artificially limit the exhalation of D_4. Any introduced D_4 will flow through the lung in a physiologically realistic manner, despite claims to the contrary. The above equation is equivalent to the tubular equilibrium lung used in the styrene model (*16*). The capture efficiency we used in both the rat and human models was similar and was only used to determine the delivered dose to the rat or human body as described in the Appendix of our paper (*1*). In the reference (*14*) cited in our paper, the delivered dose was experimentally determined by measuring the gas flow and inlet and outlet concentrations of D_4 at the rebreathing tube connections. We used the same model structure for both the rat and the human. Andersen et al. agreed that our rat inhalation model was correct because the human model had an identical structure.

In their letter, Andersen et al. also claimed that the "accumulation in the strongly bound fat compartment would always be zero." Figure 2 shows the accumulation in this compartment for F344 rats after low-dose inhalation (*13*).

It is informative to use animal data in a PBPK model to predict D_4 dose metrics in an animal body. This approach also allows the determination of the internal dose in target tissues, which can then be extrapolated to humans and correlated with the toxicity. However, models should be physiologically realistic and should not be used to predict phenomena beyond the reasonable bounds of the data by "fitting" highly restrictive cases. In an accurate model, the following problems should be avoided:

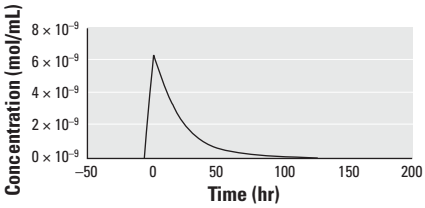

Figure 2. Accumulation of D_4 in strongly bound fat of F344 rats after low-dose inhalation.

- Artificially high pulmonary clearance of D_4 resulting from use of a $P_{b:a}$ that is not comparable to one obtained experimentally.
- Use of unconventional methods to reduce the potential of accumulation in target organs.
- Overestimation of the rate of metabolism, which is caused by a reduced absorbed dose resulting from inhalation exposure.
- Inappropriate use of the inhalation model for D_4 to examine the disposition and fate of D_4 leached from silicone breast implants.

Because of these problems with Andersen et al.'s model (*2*), the authors underestimated the potential bioavailability of D_4 and were unable to predict its bioaccumulation after repeated exposures or long-term exposure that occurs when D_4 leaches from silicone breast implants.

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